UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/723,520	11/26/2003	Mark R. Andersen	4944 US	8934

22896 7590 04/29/2008

MILA KASAN, PATENT DEPT.
APPLIED BIOSYSTEMS
850 LINCOLN CENTRE DRIVE
FOSTER CITY, CA 94404

EXAMINER	
PANDE, SUCHIRA	

ART UNIT	PAPER NUMBER
1637	

MAIL DATE	DELIVERY MODE
04/29/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/723,520	ANDERSEN ET AL.	
	Examiner	Art Unit	
	SUCHIRA PANDE	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 February 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-3,5,6,8-18,22,23,43 and 44* is/are pending in the application.

4a) Of the above claim(s) *10-18* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-3,5,6,8-9, 22-23, 43-44* is/are rejected.

7)☒ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on February 1, 2008 has been entered.

Claim Status

2. Applicant has amended claims 1, 22, 23, 43, and 44, cancelled claims 4, 7, 19-21, 24-42; and withdrawn claims 10-18. Consequently claims 1-3, 5-6, 8-9, 22-23, 43 and 44 are active and will be examined in this action.

Response to arguments

Re 103 (a) rejections of claims1-2, 4-6, 8-9, 43 and 44 over Heid et al. Ohnishi et al. and First et al.

3. Applicant's arguments filed February 1, 2008 have been fully considered but they are not persuasive. Applicant incorrectly alleges that all elements of the presently claimed invention are not taught by the cited art references.

Applicant argues that Heid et al. do not teach "ninety-five to one-thousand and thirteen oligonucleotide probes----." This was pointed out by the Examiner in the last action on page 6 of Office Action mailed on August 1, 2007 that "Heid **does not teach** "amplifying ninety-five to one-thousand and thirteen molecules----"

Ohnishi was used as the reference that taught ninety-five to one-thousand and thirteen oligonucleotide probes. Relevant section of last office action is shown below:

Regarding claims 1 and 44, Ohnishi et al. (see abstract) teach use of 200 primers (100 primer pairs) in a multiplex PCR reaction to amplify 100 genomic DNA fragments each containing a different amplified target qene sequence of interest (namely a SNP). Thus by teaching amplification of 100 samples, Ohnishi et al. teach amplifying ninety-five to one-thousand and thirteen molecules (since 100 samples taught by Ohnishi falls in the middle of the range recited in the instant claim) derived from a sample by polymerase chain reaction in the presence of a plurality of amplification primer sets suitable for amplifying target gene sequence of interest. Ohnishi et al. teaches use of allele specific probes that are labeled with FAM or VIC (see page 472, par. 3). By this teaching Ohnishi teaches 100 labeled oligonucleotides probes each of which is complementary to a region of a different amplified target qene sequence of interest. Ohnishi et al. teaches 100 primer pairs (see pages 473-474 table 1). Thus teaching (i) wherein the amplifying of step (i) comprises ninety-five to one-thousand and thirteen PCR primer pairs, wherein each primer of the ninety-five to one-thousand and thirteen PCR primer pairs.

Regarding claim 1, Ohnishi et al. teaches each primer pair is present at a concentration of 50 picomol (see page 471 par. 2).

Regarding claim 1, Ohnishi et al. do not teach each primer pair is present at a concentration of 30-45 picomolar (see page 471 par. 2).

Regarding claim 1, First et al. teach use of primer pairs in the concentration of 30-45 picomolar in multiplex PCR reactions (see Table 2 where preferred primer concentrations are taught. See primer pairs identified by SEQ ID 5 and 6 where 35 pM conc is taught; SEQ ID 21 and SEQ ID 22, SEQ ID 23 and 24 are used at 36.5 pM each; SEQ ID 97 and 98 are used at 44.5 pM each).

The prima facie case of obviousness was made by examiner by providing following statements: It would have been prima facie obvious to one of ordinary skill in the art to practice the method of Ohnishi et al. and First et al. in the method of Heid et al. at the time the invention was made. The motivation to do so is provided to one of ordinary skill by both Ohnishi et al. and First et al.

Ohnishi et al. state " To reduce the amount of DNA required to less than 1ng for the assay of a single SNP and to make genome wide-association studies feasible, we combined a multiplex PCR method with the invader assay" (see page 471 par. 3). " The reproducibility and universality of the method was confirmed with two additional sets of 100 SNPs. Because we used 40 ng of --- DNA as a template for multiplex PCR, the amount needed to assay one SNP was only 0.4 ng." (see abstract last part). This teaching explicitly tells one of ordinary skill that by using the multiplexing method of Ohnishi et al. in the method of Heid et al. miniscule amounts of mRNA containing a gene of interest would be suffice as starting material from which cDNA could be made and quantified using this method.

First et al. state "it must be noted that these primer concentrations are the preferred concentrations. Variations maybe made in the concentration of the various

primer concentrations to optimize PCR" (see col. 18, lines 25-27). Thus, an ordinary practitioner would have recognized that the primer concentration could be adjusted down to 30-45 pM each from the 50 pM each taught by Ohnishi et al. to maximize the desired results.

As noted in *In re Aller*, 105 USPQ 233 at 235,

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of specific primer concentrations for amplification was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art.

Re 103 (a) rejections of 1-4,19-28 and 32 over Dolganov et al. in view First et al.

4. Applicant argues that Dolganov et al. do not teach step (i) of the claimed method namely (i) amplifying ninety-five to one- thousand and thirteen cDNA molecules derived from a sample by polymerase chain reaction in the presence of a plurality of amplification primer sets suitable for amplifying target gene sequence of interest, and in the presence of one ninety-five to one-thousand and thirteen oligonucleotide probes complementary to a region of an amplified target gene sequence, said ninety-five to one-thousand and thirteen oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time, each of which is complementary to a region of a different amplified target ,gene sequence of interest,

Again Examiner would like to point out the relevant section of the previous office action --page 9 where this was described. That part of last office action is being reproduced below:

Regarding claim 1, Dolganov et al. teaches a method for quantifying the expression of target gene sequences of interest in a sample (see page 1473, par. 2 where real time PCR quantification is taught), comprising the steps of:

(i) amplifying ninety-five to one-thousand and thirteen cDNA (see page 1473, par. 1 where a two step process incorporating multiplex PCR (typically with a mix of 100 – 300 gene specific primer sets) followed by real time PCR on generated cDNA product is taught, thus by teaching 300 cDNA amplification Dolganov et al. teach amplifying ninety-five to one-thousand and thirteen cDNA (as 300 cDNAs and corresponding nested primer sets and probes taught by Dolganov et al. falls within the range recited in the instant claims) molecules derived from a sample by polymerase chain reaction in the presence of a plurality of amplification primer sets suitable for amplifying target gene sequence of interest, and in the presence of ninety-five to one-thousand and thirteen oligonucleotide probes complementary to a region of an amplified target gene sequence, said ninety-five to one-thousand and thirteen oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time each of which is complementary to a region of a different amplified target gene sequence of interest (See par. 2 where real time amplification PCR using 200 gene-specific primers is taught this inherently involves use of a labled probe labeled with a labeling system suitable for monitoring the amplification reaction as a function of time)

(Also see page 1474, par. 1 and Fig. 1 where RT-PCR for 34 genes was performed. Taqman hybridization probe is taught here which inherently indicates that it is complementary to a region of a different amplified target gene sequence. Taqman probe has fluorescein reporter dye at 5' end (FAM)). This along with the teaching above that Taqman primers and probes are used inherently teaches that probe used is complementary to a region of a different amplified target gene sequence of interest.

(ii) quantifying the target gene sequences amplified in step (Gene quantification via real time PCR using nested Taqman primers is taught see legend of fig. 1) .

(i) wherein the amplifying of step (i) comprises ninety-five to one-thousand and thirteen PCR primer pairs (see above),

Regarding claim 1, Dolganov et al. do not teach wherein each primer of the PCR primer pairs is present at a concentration of 30-45 picomolar.

Regarding claim 1, Regarding claim 1, First et al. teach use of primer pairs in the concentration of 30-45 picomolar in multiplex PCR reactions (see Table 2 where preferred primer concentrations are taught. See primer pairs identified by SEQ ID 5 and 6 where 35 pM conc is taught; SEQ ID 21 and SEQ ID 22, SEQ ID 23 and 24 are used at 36.5 pM each; SEQ ID 97 and 98 are used at 44.5 pM each).

The motivation to combine the references was also provided by examiner in the last action by stating "It would have been prima facie obvious to one of ordinary skill in the art to practice the method of First et al. in the method of Dolganov et al. at the time the invention was made. The motivation to do so is provided to one of ordinary skill both by Dolganov et al. and First et al.

In the section relating to Real-Time PCR Dolganov et al. state "Typically, an equivalent of 2.5 fg to 10 pg of total RNA was used in 25 μl of universal Master Mix. All forward and reverse TaqMan primers were optimized" (see page 1481 last par.). Thus Dolganov et al. teach to one of ordinary skill that optimization is performed for all primers. They do not specifically state the parameters that are optimized and the range of values associated with them.

First et al. provide specific guidance to one of ordinary skill in the art by stating "it must be noted that these primer concentrations are the preferred concentrations. Variations maybe made in the concentration of the various primer concentrations to optimize PCR" (see col. 18, lines 25-27). Thus providing explicit guidance to one of ordinary skill that the range of concentrations taught by First et al. work well for the various primers used by them and the practitioner with an ordinary skill in the art may adjust the primer concentration down to as low 30-45 pM each in multiplex PCR reactions."

Hence the rejections made earlier were valid and are being maintained.

Response to Claim Amendment

5. In the current amendment Applicant has taken limitations of former dependent claims and added them to the base claim 1, thus changing the scope of the claimed invention. New grounds of rejections are being introduced to address the amended base claim 1.

Claim Rejections - 35 USC § 103

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

8. Claims 1-3, 5-6, 8-9, 22-23,and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Unger et al. (US pat. 7,118, 910 B2 issued Oct, 10, 2006 with priority back to June 24, 2002) in view of Wang et al. (1998) Science 280: page 1077-1082; Ohnishi et al. (2001) J. Hum Genet 46:471-477; Dolganov et al. (2001) Genome Research 11:1473-1483 and First et al. (US Pat. 5,776,682 issued July 7, 1998).

Regarding claim 1, Unger et al. teach:

a method for quantifying the expression of target gene sequences of interest in a sample (see col. 22 line 41 where Quantitative RT-PCR is taught), comprising the steps of:

(i) amplifying ninety-five to one-thousand and thirteen (See col. 30 lines 17-21 where the array based device is taught for multiplexing large number of amplification reaction at the same time. By teaching multiplexing a large number, Unger et al. teach ninety-five to one-thousand and thirteen)

cDNA molecules derived from a sample by polymerase chain reaction in the presence of a plurality of amplification primer sets suitable for amplifying target gene sequence of interest (see col. 28 lines 49-50 where cDNA reverse transcribed from an mRNA is taught), and

in the presence of ninety-five to one-thousand and thirteen oligonucleotide probes complementary to a region of an amplified target gene sequence, said ninety-five to one-thousand and thirteen oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time, each of which is complementary to a region of a different amplified target gene sequence of interest (see col. 30 lines 49-55 where multiple expression analysis at each reaction site within this array device is taught using quantitative methods such as TaqMan. By teaching the TaqMan method for performing multiple expression analysis at each site Unger et al. teach oligonucleotide probes complementary to a region of an amplified target gene sequence, said oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time, each of which is complementary to a region of a different amplified target gene sequence of interest. This is because TaqMan method is a method used to perform real time PCR which requires oligonucleotide probes complementary to a region of an amplified target gene

sequence. These probes for each region have to be labeled with a labeling system suitable for monitoring the amplification reaction as a function of time—then only the real time analysis can be conducted. Since multiplex reaction is being conducted then the probes have to be complementary to a region of a different amplified target gene sequence of interest so that all the target regions of interest can be followed in the real time manner. See col. 22 lines 54-63 where details of labeled probes used in TaqMan assay are provided along with statement that both probe and PCR primers are included in the reaction mixture. Thus indicating the PCR is conducted in presence of both primers and probes for the regions to be amplified and detected using real time quantitative PCR. Both the upstream and downstream primers flanking the region of interest along with a probe complementary to target nucleic acid are taught (see col. 22 lines 59-63), and

(ii) quantifying at least one of the target gene sequences amplified in step (i). in a real-time PCR (see col. 22 lines 41-53 where details of quantitative real time PCR using TaqMan are provided) in which the product of step (i) is divided into a plurality of aliquots and said real-time PCR quantifying in step (ii) is performed on said aliquots (see col. 22 lines 41-46 where quantity of a target nucleic acid present in a sample is taught to be determined by measuring the amount of amplification product **formed after the amplification process itself**. By teaching quantification after amplification process itself and teaching multiplex amplification can be performed within single reaction site suing different pairs of primers for different targets (see col. 30 lines 25-28), Unger et al. teach the product of step (i) is divided into a plurality of aliquots and said real-time PCR

quantifying in step (ii) is performed on said aliquots. This is because the amount of each target that was present in the starting mix has to be determined from amplified mixture. So the amplified mixture from step (i) necessarily has to be divided into a plurality of aliquots (corresponding to the number of targets to be quantified) and said real-time PCR quantifying in step (ii) is performed on said aliquots so that each target that was to be quantified can be analyzed),

wherein the quantifying in step (ii) comprises PCR amplifying with at least one of the primer pairs in step (i) and at least one of the oligonucleotide probes in step (i). (Since Unger et al. teaches real time PCR as explained above that method requires that the PCR amplification takes place with the pair of primers that will amplify the target to be quantified and the probe that is specific for that given target be also present).

Regarding claim 6, Unger et al teach thermostable DNA polymerase. (See col. 25 lines 6-17)

Regarding claim 8, Unger et al teach wherein label is a fluorophore (see col. 34 lines 53-67 where FAM and VIC are taught as fluorophore label).

Regarding claim 9, Unger et al teach in which said at least one oligonucleotide probe is selected from the group consisting of 5'-exonuclease probes, stem-loop beacon probes (see col. 33 line 61 where TaqMan probe is taught—this is a 5'-exonuclease probe and see col. 23 line 37-60 where molecular beacon probes are taught).

Regarding claim 1 Unger et al. teach multiplex PCR with many targets but do not explicitly recite the number "ninety-five to one-thousand and thirteen" in context of the numbers of cDNA recites in the claim; they also do not recite this number in context of

the labeled oligonucleotide probes; similarly they do not recite this number in context of the number of primer pairs used to amplify the targets .

With regards to claim1, Unger et al. does not teach following:

express recitation of ninety-five to one-thousand and thirteen targets;

the primers flanking the above numbered targets which corresponds to ninety-five to one-thousand and thirteen primer pairs; and

ninety-five to one-thousand and thirteen labeled oligos that will be suitable probes for each of the above targets to be quantified by real time PCR method;

wherein each primer of the ninety-five to one-thousand and thirteen PCR primer pairs in step (i) is present at a concentration of 30-45 picomolar.

Regarding claim 1, Wang et al. teach detection of 2748 candidate SNPs after amplification of sample from an individual—meaning amplification of at least 2748 targets are taught from one individual sample. Each target amplification requires two primers (upstream and downstream therefore teaching 2748 pairs of primers. Therefore art teaches amplification and detection of at least 2748 targets using 2748 pairs of primers corresponding to the targets in the sample. (see whole article specially page 1078 last col. Par. 2 and 3).

Regarding claim 1, by teaching detection of 2748 targets from a sample, Wang et al. teach amplification of ninety-five to one-thousand and thirteen targets; the primers flanking the above numbered targets which corresponds to ninety-five to one-thousand and thirteen primer pairs. Since ninety-five to one-thousand and thirteen targets are taught. To quantify these many targets corresponding number of oligonucleotide probes

will be necessarily required during real time PCR. Wang et al. also teaches multiplexing (see page 1082 footnote 26 where multiplex PCR is taught.)

Regarding claims 22 & 23, Wang et al. teach a multiplexing PCR method wherein an observed efficiency of amplification is 96 % (see page 1080 par. 4 where 96% of the 512 loci assayed using multiplex PCR amplification were correctly read in 100% of individuals). Thus teaching wherein an observed efficiency of amplification is greater than 70% (claim 22) and the observed efficiency of amplification is greater than 90% (claim 23).

It would have been prima facie obvious to one of ordinary skill in the art to practice the method of Wang et al. in the method of Unger et al. at the time the invention was made. The motivation to do so is provided to one of ordinary skill by art itself (as exemplified by Ohnishi et al.) and by Dolganov et al.

Art teaches 100ng of genomic DNA is required to assay a single SNP. Therefore, only 1000SNPs can be genotyped from 100 μg of genomic DNA (equivalent to a 5- to 10 ml sample of whole blood). Hence this assay is not applicable for SNP genotyping on the very large scale needed for genome-wide association studies, for which 50,000-300,000 SNPs should be typed in each individual's DNA. To reduce the amount of DNA required to less than 1 ng for the assay of a single SNP and to make genome-wide association studies feasible, we combined a multiplex PCR method with the invader assay" (see Ohnishi et al. (2001) J. Hum Genet. 46;471-477 page 471 par. 2-3 introduction). They go on to state "We amplified 100 genomic DNA fragments, each

containing one SNP, in a single tube" (see abstract). Thus art teaches multiplexing of 100 targets in one tube.

Wang et al. teaches amplification of 2748 targets from single sample. Wang et al teaches use of multiple arrays on chips to detect these many targets.

Regarding claim 1, Dolganov et al. teaches a method for quantifying the expression of target gene sequences of interest in a sample (see page 1473, par. 2 where real time PCR quantification is taught), comprising the steps of:

(i) amplifying cDNA (see page 1473, par. 1 where a two step process incorporating multiplex PCR (typically with a mix of 100 –300 gene specific primer sets) followed by real time PCR on generated cDNA product is taught, thus by teaching 300 cDNA amplification Dolganov et al. teach amplifying ninety-five to one-thousand and thirteen cDNA (as 300 cDNAs and corresponding nested primer sets and probes taught by Dolganov et al. falls within the range recited in the instant claims) molecules derived from a sample by polymerase chain reaction in the presence of a plurality of amplification primer sets suitable for amplifying target gene sequence of interest, and in the presence of ninety-five to one-thousand and thirteen oligonucleotide probes complementary to a region of an amplified target gene sequence, said ninety-five to one-thousand and thirteen oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time each of which is complementary to a region of a different amplified target qene sequence of interest (See par. 2 where real time amplification PCR using 200 gene-specific primers is taught this inherently involves use of a labled probe labeled with a labeling system suitable for

monitoring the amplification reaction as a function of time) (Also see page 1474, par. 1 and Fig. 1 where RT-PCR for 34 genes was performed. Taqman hybridization probe is taught here which inherently indicates that it is complementary to a region of a different amplified target gene sequence. Taqman probe has fluorescein reporter dye at 5' end (FAM)). This along with the teaching above that Taqman primers and probes are used inherently teaches that probe used is complementary to a region of a different amplified target qene sequence of interest, and

(ii) quantifying the target gene sequences amplified in step (Gene quantification via real time PCR using nested Taqman primers is taught see legend of fig. 1) .

(i) wherein the amplifyinq of step (i) comprises ninety-five to one-thousand and thirteen PCR primer pairs (see above),

(ii) quantifying the target gene sequences amplified in step (i) (Gene quantification via real time PCR using nested Taqman primers is taught see legend of fig. 1) said quantifying comprises analysis by real-time polymerase chain reaction amplification (see page 1473 abstract where gene quantification via real-time PCR-based method is taught)

Dolganov et al. teaches a method in which the amplifying in step (i) is further carried out in the presence of an oligonucleotide probe complementary to a region of an amplified target gene sequence of interest, said probe being labeled with a labeling system suitable for monitoring the amplification reaction in step (i) as a function of time (see page 1474, par. 1 and Fig. 1 where RT-PCR for 34 genes was performed. Taqman hybridization probe is taught here which inherently indicates that it is complementary to

a region of an amplified target gene sequence. Taqman probe has fluorescein reporter dye at 5' end (FAM) which is suitable for real time detection see legend of fig. 1).

Dolganov et al. teaches a method in which the product of step (i) is divided into a plurality of aliquots and said quantifying in step (ii) is performed on said aliquots (see page 1474 par. 2).

Dolganov et al. teaches a method in which step (ii) comprises amplifying the product in one or more separate aliquots by polymerase chain reaction in the presence of an amplification primer set suitable for amplifying one of the target sequences of the plurality (see page 1474 par. 2 and fig. 1 where multiplex RT-PCR is followed by real time PCR).

Regarding claim 2, Dolganov et al. teaches generating RT-PCR for 34 genes as described above for claim 1 therefore it inherently teaches a method in which the amplification of step (i) is further carried out in the presence of a reverse transcriptase such that the polymerase chain reaction is reverse-transcription polymerase chain reaction and wherein the one or more cDNA molecules is obtained from mRNA derived from the sample.

Regarding claim 3, Dolganov et al. teaches a method in which the one or more cDNA molecules comprise a cDNA library (see page 1474 where 34 genes of varying abundance in the sample were reverse transcribed and the RT-PCR products were cloned into pCRII-TOPO vector is taught. Thus Dolganov et al. teaches a cDNA library).

Regarding claim 5, Dolganov et al. teaches the method wherein the polymerase chain reaction of step (i) is carried out for a number of cycles such that amplification

remains in the linear range (see page 1474 section validation of two step ---multiplex RT-PCR protocol and Fig. 2 on page 1475 where linearity of two step PCR protocol is taught. also See legend of fig. 1 on page 1474 where its taught that multiplex PCR is monitored carefully to evade plateau phase of PCR).

Regarding claim 6, Dolganov et al. teaches where the amplification in step (i) is achieved with a thermostable DNA polymerase (see page 1481 where Klen-Taq DNA polymerase is taught as a thermostable DNA polymerase).

Regarding claim 44, Dolganov et al. teach amplifying the cDNA molecules comprises as many as fourteen cycles (see fig. 6 where 10 to 25 cycles are shown. The data in graph includes amplifying the cDNA molecules comprises as many as 14 cycles).

Dolganov et al. state" we have developed a novel two-step RT-PCR approach for transcriptional profiling of multiple low –abundance mRNAs that requires significantly less starting RNA than conventional TaqMan approaches. This improved sensitivity allows transcriptional profiling in small biologic samples, such as 1-100 cells. The method relies on final gene quantification via-real time PCR using cDNA product generated by controlled hot start multiple RT-PCR approach. In contrast with conventional TaqMan approach, this method requires lower amounts of starting RNA and therefore could be applied to quantify multiple low expressed genes in small clinical samples" (see page 1477 last par.). They go on to state "the proposed method has two important advantages over conventional quantitative PCR, TaqMan assays, and gene microarrays. First, the new method allows simultaneous quantification of hundreds of

transcripts using as little as 2.5 fg of total RNA per gene whereas conventional TaqMan assays require substantially larger amounts of total RNA, usually from 10 ng to 1 mg per reaction, depending on the abundance of the genes of interest.-----Second, in contrast with gene microarray methods that have a much smaller dynamic range, lower specificity and sensitivity, real –time PCR has a dynamic range of more than six orders of magnitude and allows simultaneous accurate measurements of low- and high-abundance mRNAs." (see Dolganov et al. page 1479 last par.).

Since the number of targets claimed in the instant invention fall in the range taught by prior art as something that is feasible to amplify in multiplex reactions (Ohnishi et al. teaches amplification of 100 targets and Wang et al teaches amplification of 2748 targets). Thus by combining the method of Wang et al. in the method of Unger et al. one has a reasonable expectation of success that ninety-five to one-thousand and thirteen cDNA targets can be amplified in the microfluidic apparatus taught by Unger and quantified using the real-time method taught by Unger.

Regarding claim1 neither Unger et al. nor Wang et al. or Dolganov et al. teach wherein each primer of the PCR primer pairs in step (i) is present at a concentration of 30-45 picomolar.

Regarding claim 1, First et al. teach use of primer pairs in the concentration of 30-45 picomolar in multiplex PCR reactions (see Table 2 where preferred primer concentrations are taught. See primer pairs identified by SEQ ID 5 and 6 where 35 pM conc is taught; SEQ ID 21 and SEQ ID 22, SEQ ID 23 and 24 are used at 36.5 pM each; SEQ ID 97 and 98 are used at 44.5 pM each).

It would have been prima facie obvious to one of ordinary skill in the art to practice the method of First et al. in the method of Unger et al., Wang et al, Ohnishi et al and Dolganov et al. at the time the invention was made. The motivation to do so is provided to one of ordinary skill both by Dolganov et al. and First et al.

In the section relating to Real-Time PCR Dolganov et al. state "Typically, an equivalent of 2.5 fg to 10 pg of total RNA was used in 25 μl of universal Master Mix. All forward and reverse TaqMan primers were optimized" (see page 1481 last par.). Thus Dolganov et al. teach to one of ordinary skill that optimization is performed for all primers. They do not specifically state the parameters that are optimized and the range of values associated with them.

First et al. provide specific guidance to one of ordinary skill in the art by stating "it must be noted that these primer concentrations are the preferred concentrations. Variations maybe made in the concentration of the various primer concentrations to optimize PCR" (see col. 18, lines 25-27). Thus providing explicit guidance to one of ordinary skill that the range of concentrations taught by First et al. work well for the various primers used by them and the practitioner with an ordinary skill in the art may adjust the primer concentration down to as low 30-45 pM each in multiplex PCR reactions."

9. Claim 43 is rejected under 35 U.S.C. 103(a) as being unpatentable over Unger et al.; Wang et al.; Ohnishi et al.; Dolganov et al. and First et al. as applied to claim 1 above, and further in view of Heid et al. (1996) Genome Research 6: 986-994.

Regarding claim 43, Unger et al.; Wang et al.; Ohnishi et al.; Dolganov et al. and First et al. teach the method of claim 1, but do not teach the method in which amplification is carried out in the presence of uracil-N-glycosylase.

Regarding claim 43, Heid et al. teaches method of claim 1 in which the amplification is carried out in the presence of uracil N-glycosylase (see page 993 par. 3 where AmpErase uracil N-glycosylase is taught).

It would have been prima facie obvious to one of ordinary skill in the art to practice the method of Heid et al. in the method of Unger et al.; Wang et al.; Ohnishi et al.; Dolganov et al. and First et al. at the time the invention was made. The motivation to do so is provided to one of ordinary skill by Heid et al.

Heid et al state " we have developed a novel 'real –time" quantitative PCR method.---Unlike other quantitative PCR methods, real –time PCR does not require post-PCR sample handling, preventing potential PCR product carry over contamination and resulting in much faster and higher throughput assays" (see abstract).

Conclusion

10. All claims under consideration 1-3, 5-6, 8-9, 22-23, 43-44 are rejected over prior art.

11. Any inquiry concerning this communication or earlier communications from the examiner should be directed to SUCHIRA PANDE whose telephone number is (571)272-9052. The examiner can normally be reached on 8:30 am -5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Suchira Pande
Examiner
Art Unit 1637

/Teresa E Strzelecka/
Primary Examiner, Art Unit 1637

April 20, 2008